

[0009] FIG. 4 depicts LCMS analysis of the heterodimer purity of exemplary anti-HER2 biparatopic antibodies. FIG. 4A depicts results from LC-MS analysis of the pooled SEC fractions of v5019. FIG. 4B depicts the results from LC-MS analysis of the pooled protein A fractions of v10000.

[0010] FIG. 5 depicts analysis of a 25 L-scale preparation of an exemplary anti-HER2 biparatopic antibody. FIG. 5A depicts the SDS-PAGE profile of an exemplary anti-HER2 biparatopic following MabSelect™ and HiTrap™ SP FF purification. FIG. 5B depicts LCMS analysis of the purified antibody.

[0011] FIG. 6 compares the ability of an exemplary biparatopic anti-HER2 antibodies to bind to HER2+ whole cells displaying different HER2 receptor density compared to control antibodies, as measured by FACS. FIG. 6A and FIG. 6E depict binding to SKOV3 cells;

[0012] FIG. 6B depicts binding to JIMT1 cells; FIG. 6C and FIG. 6F depict binding to MCF7 cells; FIG. 6D depicts binding to MDA-MB-231 cells; and FIG. 6G depicts binding to WI-38 cells.

[0013] FIG. 7 depicts the ability of exemplary anti-HER2 biparatopic antibodies to inhibit the growth of HER2+ cells. FIG. 7A and FIG. 7D shows growth inhibition in SKOV3 cells; FIG. 7B shows growth inhibition in BT-474 cells; FIG. 7C shows growth inhibition in SKBR3 cells, and FIG. 7E shows growth inhibition in JIMT-1 cells.

[0014] FIG. 8 depicts the SPR binding data relating to the paratopes of an exemplary anti-HER2 biparatopic antibodies. FIG. 8A illustrates the K_D values (nM) of a monovalent anti-Her2 antibody (v1040; representing the antigen-binding domain on CH-B of exemplary anti-Her2 biparatopic antibody), for binding to immobilized Her2 ECD or dimeric Her2-Fc. FIG. 8B illustrates the K_D values (nM) of a monovalent anti-Her2 antibody (v4182; representing the antigen-binding domain on CH-A of exemplary anti-Her2 biparatopic antibody) for binding to immobilized Her2 ECD or dimeric Her2-Fc.

[0015] FIG. 9 depicts the ability of exemplary anti-HER2 biparatopic antibody to internalize in HER2+ cells. FIG. 9A depicts internalization in BT-474 cells, while FIG. 9B depicts internalization in JIMT-1 cells.

[0016] FIG. 10 depicts surface binding and internalization of exemplary anti-HER2 biparatopic antibodies. FIG. 10A (v5019) depicts the result in BT-474 cells; FIG. 10B (v5019) and FIG. 10F (v5019 and v10000) depict the result in JIMT1 cells; FIG. 10C (v5019) and FIG. 10E (v5019 and v10000) depict the result in SKOV3 cells, and FIG. 10D (v5019) depicts the result in MCF7 cells.

[0017] FIG. 11 depicts the ability of an exemplary anti-HER2 biparatopic antibody to mediate ADCC in SKOV3 cells. In FIG. 11A, the assay was carried out using an effector to target cell ratio of 5:1; in FIG. 11B, the assay was carried out using an effector to target cell ratio of 3:1; and in FIG. 11C, the assay was carried out using an effector to target cell ratio of 1:1.

[0018] FIG. 12 depicts the characterization of affinity and binding kinetics of monovalent anti-HER2 (v630 and v4182) and an exemplary biparatopic anti-Her2 antibody (v5019) to recombinant human HER2. FIG. 12A shows the measurement of k_a (1/Ms). FIG. 12B shows the measurement of k_d (1/s). FIG. 12C shows the measurement of K_D (M).

[0019] FIG. 13 depicts affinity and binding characteristics of an exemplary biparatopic anti-HER2 antibody to recom-

binant human HER2 over a range of antibody capture levels. FIG. 13A depicts the measurement of k_d (1/s) to HER2 ECD determined over a range of antibody capture levels for exemplary biparatopic anti-Her2 antibody (v5019). FIG. 13B depicts the measurement of k_d (1/s) to HER2 ECD determined over a range of antibody capture levels for monovalent anti-Her2 antibody (v4182). FIG. 13C depicts the measurement of k_d (1/s) to HER2 ECD determined over a range of antibody capture levels for monovalent anti-Her2 antibody (v630).

[0020] FIG. 14 shows a comparison of the mechanism of binding of a monospecific anti-ECD4 HER2 antibody (left), and a Fab-scFv biparatopic anti-ECD2×ECD4 HER2 antibody (right). The monospecific anti-ECD4 HER2 antibody is capable of binding one antibody molecule to two HER2 molecules; whereas the biparatopic anti-ECD2×ECD4 HER2 antibody is capable of binding one antibody to two HER2 molecule, as well as 2 antibodies to one HER2 molecule and combinations therein which results in HER2 receptor cross-linking and lattice formation followed by downstream biological effects such as internalization and/or growth inhibition as indicated by the arrows. IEC represents “immune effector cells.” The four extracellular domains of HER2 are numbered as 1, 2, 3, or 4 where 1=ECD1, 2=ECD2, 3=ECD3, and 4=ECD4.

[0021] FIG. 15 depicts the effect of an exemplary anti-HER2 biparatopic antibody on AKT phosphorylation in BT-474 cells.

[0022] FIG. 16 depicts the effect of an exemplary anti-HER2 biparatopic antibody on cardiomyocyte viability. FIG. 16A depicts the effect of v5019 and the corresponding ADC v6363 on cardiomyocyte viability; FIG. 16B depicts the effect of v5019, v7091, and v10000 and corresponding ADCs v6363, 7148, 10553 on cardiomyocyte viability, and FIG. 16C depicts the effect of v5019, v7091, and v10000 and corresponding ADCs v6363, 7148, 10553 on the viability of doxorubicin-pretreated cardiomyocytes.

[0023] FIG. 17 depicts the ability of exemplary anti-HER2 biparatopic antibody drug conjugates to inhibit the growth of HER2+ cells. FIG. 17A shows the ability of the ADC v6363 to inhibit the growth of JIMT1 cells. FIG. 17B shows the ability of the ADC v6363 to inhibit the growth of SKOV3 cells. FIG. 17C shows the ability of the ADC v6363 to inhibit the growth of MCF7 cells. FIG. 17D shows the ability of the ADC v6363 to inhibit the growth of MDA-MB-231 cells. FIG. 17E shows the ability of ADCs v6363, v10553, and v1748 to inhibit the growth of SKOV3 cells. FIG. 17F shows the ability of ADCs v6363, v10553, and v1748 to inhibit the growth of JIMT-1 cells. FIG. 17G shows the ability of ADCs v6363, v10553, and v1748 to inhibit the growth of NCI-N87 cells.

[0024] FIG. 18 depicts the effect of a biparatopic anti-HER2 antibody in a human ovarian cancer line xenograft model (SKOV3). FIG. 18A shows the effect of the antibody on mean tumor volume. FIG. 18B shows the effect of the antibody on percent survival of the animals.

[0025] FIG. 19 depicts the effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) in a human ovarian cancer line xenograft model (SKOV3). FIG. 19A shows the effect of the antibody on mean tumor volume. FIG. 19B shows the effect of the antibody on percent survival of the animals.